Muscarnic and Nicotinic Presynaptic Modulation of EPSCs in the Nucleus Accumbens During Postnatal Development

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Received 17 December 2001; accepted in final form 24 August 2002

Zhang, Liming, and Richard A. Warren. Muscarinic and nicotinic presynaptic modulation of EPSCs in the nucleus accumbens during postnatal development. J Neurophysiol 88: 3315–3330, 2002; 10.1152/jn.01025.2001. We have studied the modulatory effects of cholinergic agonists on excitatory postsynaptic currents (EPSCs) in nucleus accumbens (nAcb) neurons during postnatal development. Recordings were obtained in slices from postnatal day 1 (P1) to P27 rats using the whole cell patch-clamp technique. EPSCs were evoked by local electrical stimulation, and all experiments were conducted in the presence of bicuculline methchloride in the bathing medium and with QX-314 in the recording pipette. Under these conditions, postsynaptic currents consisted of glutamatergic EPSCs typically consisting of two components mediated by AMPA/kainate (KA) and N-methyl-d-aspartate (NMDA) receptors. The addition of acetylcholine (ACh) or carbachol (CCh) to the superfusing medium resulted in a decrease of 30–60% of both AMPA/KA- and NMDA-mediated EPSCs. In contrast, ACh produced an increase (~35%) in both AMPA/KA and NMDA receptor-mediated EPSCs when administered in the presence of the muscarinic antagonist atropine. These excitatory effects were mimicked by the nicotinic receptor agonist 1,1-diethyl-4-phenyl-piperazinium iodide (DMPP) and blocked by the nicotinic receptor antagonist mecamylamine, showing the presence of a cholinergic modulation mediated by nicotinic receptors in the nAcb. The antagonistic effects of atropine were mimicked by pirenzepine, suggesting that the muscarinic depression of the EPSCs was mediated by M1/M4 receptors. In addition, the inhibitory effects of ACh on NMDA but not on AMPA/KA receptor-mediated EPSC significantly increased during the first two postnatal weeks. We found that, under our experimental conditions, cholinergic agonists produced no changes on membrane holding currents, on the decay time of the AMPA/KA EPSC, or on responses evoked by exogenous application of glutamate in the presence of tetrodotoxin, but they produced significant changes in paired pulse ratio, suggesting that their action was mediated by presynaptic mechanisms. In contrast, CCh produced consistent changes in the membrane and firing properties of medium spiny (MS) neurons when QX-314 was omitted from the recording pipette solution, suggesting that this substance actually blocked postsynaptic cholinergic modulation. Together, these results suggest that ACh can decrease or increase glutamatergic neurotransmission in the nAcb by, respectively, acting on muscarinic and nicotinic receptors located on excitatory terminals. The cholinergic modulation of AMPA/KA and NMDA receptor-mediated neurotransmission in the nAcb during postnatal development could play an important role in activity-dependent developmental processes in refining the excitatory drive on MS neurons by gating specific inputs.

INTRODUCTION

The nucleus accumbens (nAcb) constitutes the major portion of the ventral striatum and is an important point of convergence of information originating in several limbic structures, including the prefrontal cortex (PFC), the amygdala, the hippocampus, and the midline thalamic nuclei (Groenewegen et al. 1980, 1982, 1987; Jayaraman 1985; Kelley and Domesick 1982; Kelley and Stinus 1984; Kelley et al. 1982; Kraynak et al. 1981; Newman and Winans 1980; Phillipson and Griffiths 1985). These projections, believed to be mainly glutamatergic, are thought to mediate their excitatory drive by acting on N-methyl-d-aspartate (NMDA) and AMPA/kainate (KA) glutamatergic receptors (DeFrance et al. 1985; Finch 1996; Kombian and Malenka 1994; Nicola et al. 1996; Yim and Mogenson 1982 Zhang and Warren 1999). The primary output of the nAcb is to the ventral pallidium (Hakan et al. 1992; Yang and Mogenson 1985), which is involved in the activation of voluntary movements (Heimer et al. 1994; Swerdlow and Koob 1987). This input/output organization suggests that the nAcb is an important interface between motivational and motor systems driven by the ventral pallidium (Beninger et al. 1983; Lopes da Silva et al. 1984; Mogenson et al. 1980). The nAcb is known to be involved in reinforcement aspects of behavior (Cador et al. 1991; Joseph and Hodges 1989; Grace 1992; Matthysse 1983; Snyder 1973) and could be implicated in a number of psychiatric diseases, such as schizophrenia (Csernansky et al. 1991; Grace 1992; Matthysse 1983; Snyder 1973) and Tourette’s syndrome (Braun et al. 1993; Comings 1990).

The only class of neurons that project outside the nAcb are the medium spiny (MS) neurons, which are GABAergic and account for about 95% of the neuronal population. In addition, the nAcb contains small populations of interneurons including the large aspiny (LA) neuron, which is the only known source of acetylcholine (ACh) in the nAcb (Meredith and Chang 1994; Meredith and Wouterlood 1990; Meredith et al. 1989; Phelps et al. 1985). Cholinergic systems have been implicated in fundamental aspects of human behavior including memory, motivation, and motor behavior (File et al. 1998; Gotti et al. 1997). Interest in understanding cholinergic mechanisms involved in the control and regulation of motor and higher brain functions has been growing ever since the neostriatal cholinergic system

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was postulated to play a role in the pathophysiology of several diseases. Alterations in the levels of ACh and cholinergic receptors have been linked to neurochemical and neuropsychological diseases including schizophrenia and Parkinson’s disease (Gotti et al. 1997; Lena and Changeux 1997; MacDermott et al. 1999). Selective loss of cholinergic neurons in the nAcb in schizophrenia and Alzheimer’s disease has also been demonstrated ( Holt et al. 1999; Lehéricy et al. 1989).

In the nAcb, LA neurons establish synaptic contacts with MS neurons (Contant et al. 1996) as well as with glutamatergic terminals (Meredith and Wouterlood 1990). The action of ACh is mediated by nicotinic and muscarinic receptors, which are both present in substantial amounts in the nAcb and dorsal striatum (Bernard et al. 1992; Clarke et al. 1984; Court and Perry 1995; Hersch and Levey 1995; Hersch et al. 1994; Levey et al. 1991; Schliebs and Robner 1995). Consistent with the cellular location of cholinergic muscarinic receptors (Wei et al. 1994), ACh has been found to modulate glutamatergic neurotransmission in MS neurons by acting on presynaptic muscarinic receptors (Perrault and Lopes da Silva 1994; Sugita et al. 1991) and to increase the excitability of MS neurons by acting on muscarinic postsynaptic receptors (Sugita et al. 1991; Uchimura and North 1990). The role of nicotinic receptors in modulating the activity of MS neurons has not been investigated.

It has been proposed that the major role of nicotine cholinergic receptors in the CNS, including the nAcb, is to modulate synaptic transmission by controlling neurotransmitter release rather than by exerting direct postsynaptic actions ( Gray et al. 1996; MacDermott et al. 1999; McGehee et al. 1995; Wonnacott 1997). Nicotine has been found to facilitate the release of diverse neurotransmitters, including GABA (Guo et al. 1998; Lena et al. 1993), glutamate (Fisher and Dani 2000; Girod et al. 2000; Guo et al. 1998; McGehee et al. 1995; Radcliffe and Dani 1998; Toth et al. 1993), ACh (McGehee et al. 1995), and dopamine (Auta et al. 2000; Putfarcken et al. 2000; Rapier et al. 1988, 1990; Sharples et al. 2000), and 5-HT (Reuben and Clarke 2000). Whereas nicotine can enhance glutamatergic neurotransmission, it has also been found to differentially modulate AMPA/KA and NMDA receptor-mediated synaptic transmission (Aramakis and Metherate 1998). In the striatum, including the nAcb, nicotine has been found to increase neuronal glutamate release (Kaiser and Wonnacott 2000; Reid et al. 2000; Toth et al. 1992, 1993). The presence of nicotinic receptors on glutamatergic terminals in the nAcb is also supported by the fact that glutamatergic neuronal populations known to project to the nAcb express high levels of several nicotinic receptor subunit mRNAs, whereas a comparatively low expression of these subunits is found in the nAcb itself (Quik et al. 2000; Wada et al. 1989, 1990).

The goal of the present study was to understand how ACh, through an action on both muscarinic and nicotinic receptors, modulates glutamatergic neurotransmission in the nAcb. Our findings suggest that ACh acts on both muscarinic and nicotinic presynaptic receptors to modulate glutamatergic neurotransmission, but whereas muscarinic receptor activation depresses excitation, nicotinic receptor activation enhances glutamatergic neurotransmission. Parts of the present study have appeared in abstract form (Zhang and Warren 2000).

METHODS

Slice preparation

The procedures used to obtain nAcb slice preparation have been described elsewhere (Belleau and Warren 2000). Briefly, 400-μm parasagittal slices containing the nAcb were obtained from rat pups on the day following birth (P1) up to P27. Slices were incubated for at least 1 h before recording was undertaken in a submerged-type chamber superfused with room temperature (22–25°C) artificial cerebrospinal fluid (ACSF) containing (in mM) 126 NaCl, 26 NaHCO3, 10 dextrose, 3 KCl, 1.3 MgSO4, 2.5 CaCl2, and 1.25 NaH2PO4 with a pH of 7.4 when bubbled with a gas mixture of 95% O2-5% CO2. The nAcb was visualized with a stereo microscope using the anterior commissure, the neostriatum, the septum, and the ventricles as landmarks based on Paxinos and Watson (1986).

Recording

Whole cell recording was achieved using the blind patch-clamp technique (Blaquit et al. 1989). Pipettes were pulled from thin wall borosilicate capillary glass with a P-87 micropipette puller (Sutter Instrument). The pipettes had a resistance of 3–5 MΩ when filled with a solution containing (in mM) 140 potassium gluconate, 2 MgCl2, 0.1 CaCl2, 1.1 EGTA, 10 HEPES, 2 K2-ATP (ATP), and 0.5 guanosine trisphosphate (GTP). Biocytin (0.3%) and QX-314 (2 mM; Alomone Labs) were routinely added to the recording solution to label recorded neurons and to minimize voltage-sensitive Na+ channels generating action potential, respectively. The pH of the recording solution was adjusted to 7.3 with 8N KOH solution, and its final osmolarity was adjusted to 285–290 mosmol/kg. Neurons were recorded in continuous single-electrode voltage-clamp mode with an Axoclamp 2B amplifier (Axon Instruments). The output of the amplifier was fed to a LFP 200A DC amplifier/filter (Warner Instruments) and digitized at 0.5–10 kHz with a real-time acquisition system Digidata 1200 (Axon Instruments). Data acquisition was achieved using the pClamp 6.0 software (Axon Instruments), and offline analysis was performed with pClamp 6.0 and Cambridge Electronic Design softwares. The resting membrane potential (RMP) was measured as soon as the whole cell configuration was achieved, and the offset potential, measured on withdrawal of electrode from the cell, was accounted for assuming that it drifted in a linear fashion with time from the start of the recording session. A –10-mV correction for liquid junction potential was routinely added to membrane potential measurements (Spigelman et al. 1992).

Synaptic stimulation

Excitatory postsynaptic currents (EPSCs) were evoked by means of a monopolar tungsten microelectrode placed close to the border of the nAcb, 0.5–1.0 mm away from the recording electrode. The stimuli consisted of single 0.1-ms, 3- to 6-V cathodal pulses delivered at 15-s intervals. Paired-pulse stimulation with the same parameters and separated by 50 ms were used in some experiments to distinguish between pre- and postsynaptic mechanisms. All experiments were performed with bicuculline methochloride (BMI) 10 μM present in the superfusing medium solution to block GABA A receptor-mediated synaptic currents and to isolate glutamatergic-mediated EPSCs (Zhang and Warren 1999). Under these conditions, the addition of glutamatergic antagonists completely abolished synaptic responses (e.g., Fig. 1), and in no cases did we observed evidence that the stimulus directly activated the neuron under study. In all experiments, the membrane potential was clamped on-line at –70 mV, and the EPSCs were recorded at potentials between –100 and +40 mV using incremental steps of 20 mV.

J Neurophysiol • VOL 88 • DECEMBER 2002 • www.jn.org
CHOLINERGIC MODULATION OF EPSCS IN NUCLEUS ACCUMBENS

FIG. 1. Nature of the excitatory postsynaptic current (EPSC) evoked by local electrical stimulus in the presence of bicuculline methochloride (BMI, 10 μM). A: current traces of the response evoked by single local electrical stimulus and recorded at holding membrane potentials of −40 and −100 mV before glutamatergic antagonists application (1) and during superfusion with 6-cyano-7-nitroquinoxalene-2,3-dione (CNQX; 20 μM; 2) and CNQX and 2-amino-5-phosphonovaleric acid (APV; 50 μM; 3). Recordings were obtained in a medium spiny (MS) neuron from a P3 animal. Current traces represent the average of 8 sweeps. B: current-voltage relationship of the response (I<sub>E</sub>-V<sub>m</sub>) between −120 and 20 mV. The early component was measured 9 ms after the stimulus as indicated in A. Left vertical dotted line. The late component was measured 43 ms after the stimulus as indicated in A. Right vertical dotted line.

**Pharmacological agents**

The following pharmacological agents were used: 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 20 μM); (+)-2-amino-5-phosphono-pentanoic acid (APV; 50 μM); carbachol (CCh; 50 μM); ACh (100 μM); atropine sulfate (10 μM); pirenzepine, DMPP, and mecamylamine were from Research Biochemicals International; and CCh, atropine, pirenzepine, DMPP, and from Tocris Cookson; CCh, atropine, pirenzepine, DMPP, and mecamylamine were from Research Biochemicals International; and ACh was from Sigma. Drugs, with the exception of CNQX, which was dissolved in dimethylsulfoxide (DMSO), were made up as 10 mM stock solutions in distilled water (ACh on the day of use) and diluted with external solution to stock solutions in distilled water (ACh on the day of use) and diluted before glutamatergic antagonists application (1) and during superfusion with 6-cyano-7-nitroquinoxalene-2,3-dione (CNQX; 20 μM). CNQX and APV were obtained from Tocris Cookson; CCh, atropine, pirenzepine, DMPP, and mecamylamine were from Research Biochemicals International; and ACh was from Sigma. Drugs, with the exception of CNQX, which was dissolved in dimethylsulfoxide (DMSO), were made up as 10 mM stock solutions in distilled water (ACh on the day of use) and diluted with external solution to final concentration just before their addition to the perfusion medium. The final concentration of DMSO during CNQX administration was 0.1%. Under our experimental conditions, the full effect of cholinergic agonists on the response occurred 5–7 min following their addition to the bathing medium and no recording was made before a drug had been perfused for at least 15 min. Antagonists were added to the superfusing medium at least 15 min. and then a baseline was recorded before the addition of agonists. In several cases atropine was present in the ACSF throughout the experiment. The slice was superfused with control ACSF for at least 30 min to allow washout of a drug before a new baseline was recorded. In some experiments, slices were incubated for 2 h in the presence of the antagonist (mecamylamine) prior to experimentation to enable full penetration of the drug into the slice. Synaptic currents were stored as the on-line average of four to eight events at each membrane potential before, during, and after drug administration.

**Statistics**

Statistical analysis was performed with SigmaStat software (SPSS) using paired Student’s t-test to compare the response before and during the application of agonists and antagonists. Probability values of <0.05 were considered statistically significant. All numerical data are expressed as means ± SE. Neurons that could not be unambiguously classified as MS based on their physiological characteristics (Belleau and Warren 2000) and morphological appearance were excluded from statistical analysis.

**RESULTS**

Whole cell voltage-clamp recording was obtained from 127 MS neurons in slices from rats between P1 and P27. Most cells (n = 86) were recorded in preparations from P5 to P15 animals, a time frame during which relatively large NMDA-mediated responses can be more readily evoked (Zhang and Warren 1999). The membrane and firing characteristics of MS neurons were similar to those previously reported for animals of comparable age (Belleau and Warren 2000). In addition, 79 neurons filled with biocytin were examined under light microscopy and displayed features that have been previously attributed to MS neurons from animals of similar age (Tepper et al. 1998).

**Characteristics of glutamatergic EPSCs**

Typically, postsynaptic currents evoked by local electrical stimulation in the presence of the GABA<sub>A</sub> receptor antagonist BMI consisted of a compound glutamatergic EPSC comprising an early and a late component mediated, respectively, by the activation of AMPA/K<sub>A</sub> and NMDA receptors (Fig. 1). We characterized postsynaptic EPSCs in 91 neurons; the EPSC in 79 displayed an early and a late component, whereas only an early component was found in the remaining 12.

The early EPSC peaked between 3.6 and 21 ms after stimulus onset at a holding membrane potential of −100 mV, had a linear relationship with the membrane potential and reversed around 0 mV (n = 91). In contrast, the maximal amplitude of the late EPSC occurred much later, was usually observed at holding membrane potentials of −20 or −40 mV, displayed a nonlinear relationship with voltage, and also reversed around 0 mV (n = 79).

Figure 1 shows a representative example of an EPSC recorded in a preparation from a P3 animal on which specific glutamatergic antagonists were tested. During the control period (Fig. 1A1), the early EPSC peaked 9 ms after the stimulus onset at a holding membrane potential of −100 mV, and the response decayed to baseline within 35 ms. The current voltage relationship (I<sub>E</sub>-V<sub>m</sub>) of the early EPSC was linear at membrane potentials between −80 and 20 mV, but the response appeared to saturate at membrane potentials below −80 mV (Fig. 1B1). Bath application of the AMPA/K<sub>A</sub> receptor antagonist CNQX completely abolished the early component of the EPSC, and there was virtually no residual postsynaptic current at all membrane potentials at the latency the early response was measured (Fig. 1, A2 and B1).

The late component, measured after the early component had decayed, increased at membrane potentials between −100 and −40 mV and reached its maximum usually at −40 or −20 mV. At more depolarized membrane potentials, it decreased and reversed polarity around 0 mV (Fig. 1, A and B, 2), a current-voltage relationship typical of NMDA receptor-mediated current. The further addition of the NMDA receptor antagonist APV to the superfusing medium completely abol-
ished the late EPSC (Fig. 1A3), demonstrating that it was mediated by NMDA-type receptors. In the presence of CNQX alone, the NMDA receptor-mediated EPSC was recorded in isolation showing that measurements of the late component of the EPSC made on the compound EPSC were close to the peak of the NMDA-mediated EPSC and represented mostly NMDA receptor-mediated current (Fig. 1A2). Also, note there was no residual postsynaptic current in the presence of CNQX and APV, showing that glutamatergic EPSCs were effectively isolated by the addition of BMI to the superfusing medium. CNQX and APV were tested together in four other neurons producing similar results. In addition, CNQX and APV were tested individually in 17 and 14 neurons, respectively, producing an inhibition of the early and late components of the response of $91 \pm 2$ and $85 \pm 5\%$.

In most neurons, the effects of cholinergic agonists and antagonists were assessed at holding membrane potentials usually between $-100$ and $+40$ mV in steps of $20$ mV. The AMPA/KA-mediated EPSC was measured at the peak of the early component of the EPSC at a holding membrane potential of $-100$ mV, when the amplitude of the late component was minimal (Fig. 1A, left vertical dotted lines), whereas the effects on NMDA receptor-mediated currents were measured at a latency at which the early component recorded at a holding membrane potential of $-100$ mV had decayed (Fig. 1A, right vertical dotted lines).

Effects of cholinergic agonists

The addition of the general cholinergic agonists ACh or CCh to the superfusing medium in the presence of BMI typically produced a decrease of both the early and late components of the EPSC. A representative example of this effect is shown in Fig. 2A. In this case, the amplitude of the early and late component of the EPSC recorded at $-100$ and $-20$ mV, respectively, was reversibly reduced by $38$ and $40\%$ during the application of CCh. Similar results were observed in 15 other neurons, while CCh produced no effects on the EPSC in one case. The effects of CCh on the early and late components of the EPSC as a function of holding membrane potential are summarized in Fig. 2B. The amplitude of the early component of the EPSC was significantly reduced at holding membrane potentials between $-100$ and $-20$ mV by an average of $39-46\%$ ($n = 16$). The magnitude of the effect of CCh on the early component of the EPSC did not vary significantly with holding membrane potential ($F_{1,16} = 0.220, P = 0.926, df = 4$). No significant changes were observed at more positive membrane potentials because the EPSCs were small and the amplitudes were more variable. CCh also produced a reduction of

![FIG. 2. Effect of cholinergic agonists on the EPSC.](http://jn.physiology.org/)

**FIG. 2.** Effect of cholinergic agonists on the EPSC. A: current traces of the response evoked by single local electrical stimulus recorded at holding membrane potentials of $-20$ and $-100$ mV before ($I$), during ($I$), and after ($I$) superfusion with carbachol (CCh, 50 μM). $I$: the overlay of the responses before and during CCh application. Current traces represent the average of 8 sweeps. BMI (10 μM) was present in the superfusing medium throughout recording. Recordings were obtained from a MS neuron in a preparation from a P8 animal. Left and right vertical arrows in $I$ indicate where the early and late responses were respectively measured. B: average $I_{e}V_{m}$ of the early ($n = 16; I$) and late ($n = 14; 2$) recorded before and during superfusion with CCh. C: average $I_{e}V_{m}$ of the early ($n = 28; I$) and late ($n = 26; 2$) components of the EPSC recorded before and during superfusion with acetylcholine (ACh). In 2 neurons tested with CCh and 2 tested with ACh, the EPSC consisted only of an early component, and these were included in the average of the early component. The $I_{e}V_{m}$ of the late response were aligned on the holding membrane potential at which the response was maximum before averaging (usually at $-20$ or $-40$ mV). Asterisk indicates a statistically significant difference between control and agonist treatment at this holding membrane potential (Student’s $t$-test, $P < 0.05$).
the late component of the EPSC, which generally appeared to be of larger magnitude than that observed on the early component, averaging 45–72% (n = 14) at holding membrane potentials at which a statistically significant effect was observed. Indeed, the effect of CCh was statistically smaller on early responses recorded at −100 mV than on late responses measured at their maximal amplitude (inhibition of 45 ± 5 and 66 ± 6%, respectively; t = 3.408, P = 0.002, df = 28).

ACh (100 µM) was tested in 33 neurons; it produced a reduction of the EPSC in 28 cells and no change in the remaining 5. The inhibitory effects of ACh appeared smaller than those produced by CCh on the early component of the EPSC (Fig. 2C1), ranging from 29 to 38% (n = 28), although the difference was not statistically significant at any membrane potential (0.418 ± t = 0.667, 0.1 ± P ≤ 0.678, df = 42). As observed for CCh, the magnitude of the effects of ACh did not vary significantly with holding membrane potential (F = 0.700, P = 0.594, df = 4). The effects of ACh on the late component of the EPSC ranged from 26 to 44% at membrane potentials at which a significant inhibition was observed (Fig. 2C2). No significant difference was found between the effect of ACh on the late component at the membrane potential at which the response was largest and on the early component recorded at −100 mV of the EPSC (inhibition of 44 ± 4 and 38 ± 5%, respectively; t = 0.866, P = 0.396, df = 52), although the effect of ACh on the late component of the response was significantly smaller than that produced by CCh (t = 2.779, df = 38, P = 0.008; 44 vs. 68%, respectively) at the membrane potential at which the late EPSC was largest.

To validate our experimental assumption that the effects of cholinergic agonists on the early and late components of the EPSC accurately represented the effects on AMPA/KA and NMDA receptor-mediated EPSCs, we studied the effects of ACh on pharmacologically isolated AMPA/KA and NMDA mediated EPSC using APV (50 µM) and CNQX (20 µM), respectively. In these experiments, ACh produced an inhibition on AMPA/KA and NMDA receptor-mediated EPSCs of a magnitude comparable to the effects observed on the early and late components of the compound EPSC. The AMPA/KA receptor-mediated EPSC peak was reduced by 40–45% at membrane potentials between −100 and −20 mV (n = 7; Fig. 3A) and the NMDA receptor-mediated current by 61% at the membrane potential at which the response was the largest (n = 4; Fig. 3B), thus confirming the observations made on compound EPSCs. In general, the effects of CCh and ACh were fully reversible after 10–30 min of washing with control ACSF.

Together, these results indicate that the activation of cholinergic receptors results in a net depression of both AMPA/KA and NMDA receptor-mediated EPSCs in nAcb MS neurons, whereas in some cases, the inhibition appeared larger on the NMDA than on the AMPA/KA mediated response.

**Effects of muscarinic receptor antagonists**

To identify the type of receptors mediating the inhibitory action of cholinergic agonists, ACh was administered along with specific cholinergic receptor antagonists. We first tested the effects of the general muscarinic receptor antagonist atropine (10 µM). When administered alone, atropine produced an increase in both the early and late components of the EPSC in five cells tested (Fig. 4A), suggesting that endogenous ACh produced a significant inhibition of the EPSC in our preparation. Interestingly, when concomitantly applied with atropine, ACh produced a further enhancement of the EPSC in most neurons tested instead of a decrease, as observed when general cholinergic agonists were administered alone.

Figure 4B shows an example of the effects produced by ACh administered in the presence and absence of atropine. In this case, atropine was first added to the superfusing medium for 15 min (Fig. 4B1) and, when ACh was added, a significant enhancement of both the early and late components of the EPSC was observed (Fig. 4B2). Following the washout of atropine, the same dose of ACh produced a significant decrease of the EPSC (Fig. 4B3) as compared with the atropine period and following the washout of ACh (Fig. 4B4).

The effects of ACh in the presence of atropine were tested in 18 neurons; a significant enhancement of the early component of the EPSC averaging 33% was observed in 13 (72%) neu-
rons, whereas ACh in the presence of atropine produced no significant change in the remaining 5 (Fig. 4C). Similarly, the late component of the response was increased by an average 36% (at the membrane potential at which the response was largest) in eight of nine neurons tested, with no significant change observed in the remaining cell. Because ACh alone reduced the amplitude of evoked EPSCs while producing an increase when given in combination with atropine, we concluded that muscarinic receptor activation mediated inhibitory effects that masked an excitation possibly mediated by nicotinic receptors.

To identify the pharmacological type of muscarinic receptors mediating the inhibitory effects of cholinergic agonists, we tested the effect of CCh in the presence of the M₁/M₄ receptor antagonist pirenzepine. In three of four cases, CCh applied in the presence of pirenzepine (10 μM) produced no effect on the early component of the EPSC (110 ± 10%) while it produced an increase of the early response of 70% in the remaining case. Following washout of pirenzepine (103 ± 12% of control), CCh alone produced a significant decrease in all four cells relative to control (−33 ± 5%). CCh in the presence of pirenzepine produced similar effects on the late response: an increase was observed in two cells (24 and 31%, respectively), but no significant change in a third one. Following washout of pirenzepine (111 ± 14% of control), CCh alone produced a decrease in the late response of 36–53% in the three neurons tested. In conclusion, pirenzepine appeared to mimic the antagonistic effects of atropine, suggesting that the M₁/M₄ receptor mediated much of the inhibitory effects of cholinergic agonists.

Effects of the nicotinic receptor agonist

To corroborate the existence of nicotinic receptor-mediated modulation of excitatory neurotransmission in the nAcb, we tested the specific nicotinic agonist DMPP (10 μM) with atropine (10 μM) present in the bathing medium throughout the experiments. DMPP was tested on both the composite EPSC (n = 9) and pharmacologically isolated AMPA/KA (n = 7) and NMDA (n = 4) receptor-mediated responses. Data from the two types of experiments were combined because similar results were obtained. As shown in a characteristic example in Fig. 5A, DMPP produced an enhancement of the EPSC that was similar to the one observed with ACh and CCh administered in the presence of atropine. DMPP increased the amplitude of the early AMPA/KA component of the EPSC in 13 of the 16 neurons (81%) and that of the late component in 12 of 13 neurons (92%) by an average of 37 ± 4% (19–80%) and 59 ± 8% (18–107%), respectively. Results are summarized in Fig. 5B. Statistically significant effects were observed at membrane potentials below −40 mV for the early response and only at the membrane potential at which the late response was maximal for the late responses. The effect of DMPP was statistically larger on the maximum of the late component of
the EPSC than on the early one recorded at a holding membrane potential of −100 mV (t = 2.423, df = 23, P = 0.024). DMPP was always administered in the presence of atropine, showing that the enhancements were independent of muscarinic mechanisms. In addition, DMPP produced no changes in either the early or late components of the EPSC in four neurons when administered in the presence of the nicotinic receptor antagonist mecamylamine (Fig. 5C).

**Effects of ACh as function of postnatal age**

We have recorded neurons from P1 to P27 animals, but most cells were recorded in preparations from P5 to P15 animals, and only with ACh we recorded a significant number of neurons over a range of postnatal ages sufficient to perform a developmental analysis (P3–P15, n = 33). The magnitude of the inhibition produced by ACh as a function of postnatal age is presented in Fig. 6 for both the early and late responses. The effects of ACh on the early component of the EPSC did not change with postnatal age, but those on the late component increased significantly during the first two postnatal weeks. In the same group of neurons, we found no statistically significant changes in the amplitude of either the early (r = 0.135, df = 26, P = 0.492) or late (r = 0.073, df = 24, P = 0.721) component of the EPSC.

**Locus of the cholinergic modulations of evoked EPSCs**

To identify the locus (pre- or postsynaptic) of action of cholinergic agonists, we compared several features of our recordings in the presence and absence of cholinergic agonists. Our evidence suggests that the effects produced by both muscarinic and nicotinic agonists were exclusively mediated by presynaptic mechanisms in the present study. First, we observed that ACh, DMPP, or ACh in the presence of atropine did not consistently produce changes in the holding membrane current at holding membrane potentials of −40 and +40 mV (Fig. 7), suggesting that cholinergic agonists produced no change in input conductance. Similar results were obtained using steady-state current-voltage curves generated by slow voltage ramps between −100 and +40 mV (not shown). Second, ACh produced no change in the decay time.
No statistically significant correlation was found between the magnitude of the effects of ACh and postnatal age ($r = 0.265, df = 26, P = 0.172; \cdots$). In contrast, during the application of DMPP ($70 \mu M$) under these conditions. In this case, CCh produced a membrane depolarization of 21 mV. Intracellular depolarizing current pulse that was subthreshold during control readily evoked spiking when CCh was added to the bath, and the number of action potentials increased in response to suprathreshold current injection. These results suggest that the presence of QX-314 into the recording pipette occluded the postsynaptic effects mediated by muscarinic receptors.

**DISCUSSION**

We have studied the effects of cholinergic agonists on isolated EPSCs in nAcb MS neurons. Broad-spectrum cholinergic agonists produced a direct effect on the membrane and/or firing properties of MS neurons when QX-314 was omitted from the pipette solution. Figure 8D shows an example of the effects produced by CCh ($50 \mu M$) under these conditions. In this case, CCh produced a membrane depolarization of 21 mV. Intracellular depolarizing current pulse that was subthreshold during control readily evoked spiking when CCh was added to the bath, and the number of action potentials increased in response to suprathreshold current injection. These results suggest that the presence of QX-314 into the recording pipette occluded the postsynaptic effects mediated by muscarinic receptors.

**FIG. 6.** Effects of ACh as a function of postnatal age. A: magnitude of the inhibitory effects of ACh on the early component of the EPSC recorded at a holding membrane potential of $-100$ mV as a function of postnatal age ($n = 28$). No statistically significant correlation was found between the magnitude of the effects of ACh and postnatal age ($r = 0.265, df = 26, P = 0.172; \cdots$). B: magnitude of the inhibitory effects of ACh on the late component of the EPSC at the holding membrane potential at which the response was maximal as a function of postnatal age ($n = 26$). A statistically significant correlation was found between the magnitude of the effects of ACh and postnatal age ($r = 0.556, df = 24, P = 0.003; \cdots$). ●, the effect observed for individual neuron in A and B.

(τ) of the evoked EPSC measured by fitting a single exponential to the isolated AMPA response (i.e., recorded in the presence of APV) at holding membrane potential of $-100$ mV ($12.2 \pm 1.4$ and $13.2 \pm 1.9$ ms during control and during ACh administration, respectively, $n = 7, P > 0.4$) or $-40$ mV ($12.6 \pm 1.3$ and $13.3 \pm 1.6$ ms during control and during ACh administration, respectively, $n = 7, P > 0.7$). Third, we used a paired-pulse protocol with a 50-ms interval between stimuli to discriminate between pre- and postsynaptic mechanisms (d’Alcatara et al. 2001; Hoffman and Lupica 2001; Mulder et al. 1996, 1997; Pennartz et al. 1991; Robbe et al. 2001; Zucker 1989). We found that the ratio (PPR; 2nd EPSC amplitude/1st EPSC amplitude) significantly changed during administration of agonists, thus suggesting presynaptic mechanisms. In the presence of ACh ($n = 7$), the amplitude of the first and second evoked EPSCs both decreased, but the second response decreased to a larger extent, resulting in a decrease in the PPR (Fig. 8A). In contrast, during the application of DMPP ($n = 6$), the amplitude of evoked EPSCs increased but the second response increased more, resulting in an increase in the PPR (Fig. 8B). Fourth, we found that ACh produced no effects on the response evoked by pressure ejection of glutamate in the vicinity of neurons in the presence of tetrodotoxin (TTX; Fig. 8C). Together, these results suggest that under the present experimental conditions muscarinic and nicotinic receptors agonists produced no detectable postsynaptic effects in the nAcb and that the present results reflect an action on presynaptic receptors.

Several studies in the nAcb and dorsal striatum have described direct postsynaptic effects on the passive and/or active membrane properties of MS neurons mediated by muscarinic receptors (Gabel and Nisenbaum 1999; Galarraga et al. 1999; Hsu et al. 1996, 1997; Pineda et al. 1995; Sugita et al. 1991; Uchimura and North 1990; see also Pennartz and Lopes da Silva 1994) consistent with the distribution of muscarinic receptor (Bernard et al. 1992; Weiner et al. 1990; Yan and Surmeier 1996). Several factors could explain the discrepancies between these studies and the present results, including the fact that we used whole cell patch clamp recording and that experiments were performed at room temperature. We found that under the present experimental conditions, cholinergic agonists produced a direct effect on the membrane and/or firing properties of MS neurons when QX-314 was omitted from the pipette solution. Figure 8D shows an example of the effects produced by CCh ($50 \mu M$) under these conditions. In this case, CCh produced a membrane depolarization of 21 mV. Intracellular depolarizing current pulse that was subthreshold during control readily evoked spiking when CCh was added to the bath, and the number of action potentials increased in response to suprathreshold current injection. These results suggest that the presence of QX-314 into the recording pipette occluded the postsynaptic effects mediated by muscarinic receptors.

**FIG. 7.** Effects of cholinergic agonists on holding membrane currents. A: average $I_{max}$ before and during superfusion with ACh ($100 \mu M$). B: same as A but during superfusion of atropine and during superfusion with DMPP or ACh in the presence of atropine. No statistically significant differences were found at any holding membrane potentials.
agonists ACh and CCh produced a reduction of both AMPA/Kα and NMDA receptor-mediated components of the EPSC. In contrast, in the presence of the muscarinic receptor antagonist atropine, cholinergic agonists produced an increase of the EPSC, suggesting that the inhibition of the EPSC was mediated by muscarinic cholinergic receptors and that, under the present experimental conditions, an excitatory effect mediated by nicotinic cholinergic receptors was masked by muscarinic-mediated inhibition. The effects of atropine were generally mimicked by the antagonist pirenzepine, suggesting that the inhibition of the EPSC by cholinergic agonists was mediated by muscarinic cholinergic receptors and that, under the present experimental conditions, an excitatory effect mediated by nicotinic cholinergic receptors was masked by muscarinic-mediated inhibition. The inhibitory effects of cholinergic agonists were mediated in the vicinity of the recorded neurons in the presence of TTX, showing that the cholinergic agonists were acting on presynaptic receptors, probably located on glutamatergic terminals. Because cholinergic agonists produced direct effects on the membrane and firing properties of MS neurons when QX-314 was omitted from the pipette solution, intracellular recordings as the other neurons in the present study with the exception that QX-314 was omitted from the pipette recording solution. Recordings were obtained under the same experimental conditions as the other neurons in the present study with the exception that QX-314 was omitted from the pipette solution. Intracellular superfusing medium. In this case, the addition of carbachol to the superfusing medium could constitute an interesting pharmacological tools for studying presynaptic mechanisms. Together, these results suggest that ACh modulates glutamatergic neurotransmission by decreasing glutamate release via an action on presynaptic muscarinic receptors or by increasing glutamate release via nicotinic receptors. These contrasting effects of ACh on single neurons emphasizes the complexity of cholinergic modulation of glutamatergic neurotransmission in the nAcb. In addition to its presynaptic effects on glutamatergic neurotransmission, ACh produces a direct modulation of the membrane and firing properties of MS neurons and is also known to modulate the release of other neurotransmitters in the nAcb. We suggest that ACh may play an important role in the nAcb by gating glutamatergic excitiation. This function may be important for synapse formation and consolidation during postnatal develop-

**FIG. 8.** Locus of the effects of cholinergic agonist on EPSCs. A: effects of ACh on PPR. Current traces of the responses evoked by a pair of single local electrical stimuli 50 ms apart at holding membrane potentials of −100 mV before (1) and during (2) superfusion with ACh (100 μM). 3: the overlay of the responses before and during ACh application; the amplitude of the 1st response in the presence of ACh was scaled to match the amplitude of the 1st response during control. Note that the 2nd response proportionally decreased more than the 1st response in the presence of ACh and that there is no apparent changes in the time course of the EPSCs. Current traces represent the average of 8 sweeps and BMI (10 μM) was present in the superfusing medium throughout recording. A: the average amplitude of the PPR from 7 neurons before and during superfusion with ACh. PPR was statistically larger in the presence of ACh than during control condition (t = 3.045, df = 6, P = 0.023). B: effects of DMPP on PPR. Conventions are the same as in A. Note that the 2nd response proportionally increased more than the 1st response in the presence of DMPP and that there is no apparent changes in the time course of the EPSCs. PPR was statistically larger in the presence of DMPP than during control condition (t = 3.660, df = 5, P = 0.015). C: current traces of the response evoked by local pressure ejection of glutamate (1 mM) from a patch pipette before (1) and during (2) superfusion with ACh (100 μM) at a holding membrane potential of −100 mV in the presence of tetrodotoxin (1 μM) and BMI (10 μM). 3: the overlay of the responses recorded in 1 and 2. 4: the amplitude of the peak response recorded at a holding membrane potential of −100 mV for 3 neurons before and during superfusion with ACh. D: effect of the cholinergic agonist carbachol on the membrane and firing properties of MS neurons. Voltage responses evoked with intracellular current pulses of 15 (top) and 75 pA (bottom) from resting membrane potential before (1), during (2), and after (3) the addition of carbachol (50 μM) to the superfusing medium. In this case, the addition of carbachol produced a 21-mV depolarization of the membrane potential. Recordings were obtained under the same experimental conditions as the other neurons in the present study with the exception that QX-314 was omitted from the pipette solution.
ment as well as in controlling MS neurons membrane bistability in mature nAcb.

**Locus of cholinergic receptors**

Our results suggest that both muscarinic depression and nicotinic potentiation of EPSCs were mediated by an action on cholinergic receptors located on glutamatergic terminals. We found that neither CCh and ACh or DMPP altered the input conductance nor changed the time course (the rise or decay phase) of EPSCs. Cholinergic agonists also produced consistent changes in the paired-pulse ratio in agreement with an action mediated by presynaptic mechanisms (d’Alcatura et al. 2001; Hoffman and Lupica 2001; Mulder et al. 1996, 1997; Pennartz et al. 1991; Robbe et al. 2001; Zucker 1989). Furthermore, ACh produced no effects on the response evoked by exogenous glutamate during blockade of synaptic transmission with TTX. In contrast, CCh produced marked effects on the membrane and firing properties of MS neurons when QX-314 was omitted from the pipette recording solution. We conclude that the modulation of EPSCs by cholinergic agonists was mediated by presynaptic mechanisms. Our findings are in general agreement with studies on the effects of muscarinic agonists on glutamatergic neurotransmission in both the nAcb (Pennartz and Lopes da Silva 1994; Sugita et al. 1991) and the dorsal striatum (Akaike et al. 1988; Barral et al. 1999; Hernandez-Echeagaray et al. 1998; Hsu et al. 1995; Malenka and Kocsis 1988), whereas, to our knowledge, there have been no reports on the modulation of glutamatergic neurotransmission by nicotinic receptor in either structure.

In agreement with Pennartz and Lopes da Silva (1994), we observed no changes in the passive membrane properties of MS neurons in the presence of cholinergic agonists, whereas several studies on the nAcb and dorsal striatum have described direct postsynaptic effects on the passive and/or active membrane properties of MS neurons mediated by muscarinic receptors. These observations suggest that postsynaptic activation of muscarinic receptors enhanced the excitability of MS neurons by producing membrane depolarization (Hsu et al. 1996; Sugita et al. 1991; Uchimura and North 1990) and an increase in input resistance (Galarraga et al. 1999; Hsu et al. 1996; Pineda et al. 1995; Uchimura and North 1990) likely by reducing K+ conductances including inward rectifying (I_Ki) and persistent (I_Kp) (Gabel and Nisenbaum 1999; Galarraga et al. 1999; Hsu et al. 1996, 1997; Pineda et al. 1995). These results are consistent with the membrane potential depolarization we observed when QX-314 was omitted from the pipette solution that likely resulted from the suppression of these K+ conductances. Postsynaptic effects mediated by muscarinic receptors were typically blocked by pirenzepine and attributed to the activation of M1 receptors. These observations are consistent with the distribution of muscarinic receptors in the nAcb and striatum where M1 receptors are primarily found postsynaptically on MS neurons (Bernard et al. 1992; Weiner et al. 1990; Yan and Surmeier 1996).

Because muscarinic receptors are coupled to G protein (Caulfield and Birdsall 1998), one possibility is that by using a whole cell recording technique, we washed out some elements of the second-messenger system necessary for the expression of postsynaptic effects even though ATP and GTP were always included in the pipette solution. Alternatively, we routinely added QX-314 to the recording pipette solution to block action potential generation. We found that by omitting QX-314 from the pipette recording solution, cholinergic agonists modulated the membrane and firing properties of MS neurons, suggesting that QX-314 occluded the postsynaptic effects of cholinergic agonists. In addition to blocking voltage-gated Na+ channels, QX-314 is also known to inhibit G-protein-gated K+ conductances (Alreja and Aghajanian 1994; Andrade 1991; Lambert and Wilson 1993; Nathan et al. 1990; Otis et al. 1993; Slesinger 2001) and may have occluded muscarinic postsynaptic effects on K+ conductances (Gabel and Nisenbaum 1999; Galarraga et al. 1999; Hsu et al. 1996, 1997; Pineda et al. 1995). This hypothesis is consistent with recent findings showing that intracellular QX-314 blocks muscarinic M1 and M3 receptor signaling pathways expressed in Xenopus oocytes (Hollmann et al. 2000, 2001). The present results suggest that internal QX-314 may also block the signaling pathway of native muscarinic receptors and that it could be a useful pharmacological tool to isolate presynaptic mechanisms in the study of the muscarinic cholinergic system or other neurotransmitter systems modulating G-protein-gated K+ conductances. Further studies would be needed to test these hypothesis.

To our knowledge, this is the first study reporting a modulation of glutamatergic neurotransmission mediated by nicotinic receptors in the nAcb or other neostriatal structures. Some of the previous studies on the nAcb and dorsal striatum have limited their scope to muscarinic receptor-mediated modulation of excitatory neurotransmission (Barral et al. 1999; Calabresi et al. 1998; Hernandez-Echeagaray et al. 1998; Sugita et al. 1991). In studies in which general cholinergic agonists were used, none reported an increase in excitation neurotransmission in the presence of muscarinic antagonists (Hsu et al. 1995; Pennartz and Lopes da Silva 1994), whereas Akaike et al. (1988) found that nicotine produced no effect on excitatory postsynaptic potentials (EPSPs) in the caudate nucleus of adult rats.

The presence of functional presynaptic nicotinic receptors in the nAcb and dorsal striatum has been documented (see Lendvai and Vizi 1999; MacDermott et al. 1999). Recent studies in other regions of the CNS have found that nicotinic agonists potentiated glutamatergic neurotransmission presumably by acting on presynaptic receptors located on glutamatergic terminals (Aramakis and Metherate 1998; Gil et al. 1997; Girod et al. 2000; Gray et al. 1996; Radcliffe and Dani 1998; McGhee et al. 1995; Vidal and Changeux 1993) in agreement with the present findings. Nicotine has also been found to exert direct postsynaptic excitation on some specific neuronal populations, including interneurons in the cerebral cortex (McCormick and Prince 1986; Porter et al. 1999; Roerig et al. 1997) and hippocampus (Frazier et al. 1998; Jones and Yakel 1997; McQuiston and Madison 1999), dopaminergic neurons in the ventral tegmental area (Calabresi et al. 1989; Pidoplichko et al. 1997), retinal ganglion cells (Feller et al. 1996), and in brain stem nucleus ambiguus (Zhang et al. 1993), but we found no evidence for a similar action in nAcb MS neurons. Nicotinic receptors are ligand-gated channels independent of second-messenger system and would not be occluded by QX-314 in the same way as muscarinic receptors.
**Muscarinic depression of EPSCs**

Few studies have examined the modulatory role of ACh on glutamatergic neurotransmission in the nAcb. Pennartz and Lopes da Silva (1994) reported that in ventral striatal slices muscarine and CCh reversibly attenuated the EPSP through presynaptic mechanisms and that this action was completely antagonized by atropine or pirenzepine in agreement with our findings. They also found that increasing endogenous levels of ACh with acetylcholinesterase inhibitors resulted in a decrease in the EPSP in accordance with our finding that endogenous ACh exerted a tonic depression of EPSC, as suggested by the increase in the EPSP produced by atropine alone in our preparation. Sugita et al. (1991) also reported that cholinergic muscarinic receptor activation depressed glutamatergic neurotransmission in the nAcb through presynaptic mechanisms.

Comparable results have been obtained in the dorsal striatum, a structure that shares several anatomical and physiological characteristics with the nAcb and in which cholinergic and muscarinic agonists have been found to decrease the responsiveness of MS neurons to excitatory inputs, presumably by acting on presynaptic muscarinic receptors (Akaile et al. 1988; Barral et al. 1999; Hernandez-Echeagaray et al. 1998; Hsu et al. 1995; Malenka and Kocsis 1988). Therefore our findings that muscarinic receptors depressed glutamatergic EPSCs by acting on presynaptic receptors are in general agreement with previous studies.

Previous studies on the nAcb and dorsal striatum made no attempt to examine the possibility that cholinergic agonists exerted differential modulation of AMPA/KA and NMDA receptor-mediated excitation. We found that the activation of muscarinic receptors depressed both AMPA/KA- and NMDAmediated EPSCs and that with CCh the depression was larger on the NMDA than on the AMPA/KA receptor-mediated component. The larger depression of the NMDA-mediated response could be the result of rundown of the NMDA response in vitro, but the fact that we observed a larger increase in the NMDA receptor-mediated component than on the AMPA/KA-mediated response with nicotinic agonists suggests that this was not the case. Alternatively, it is possible that the effects are mediated by different types of muscarinic receptors for which ACh and CCh have different binding characteristics.

There are no highly selective antagonists for muscarinic receptor subtypes (Caulfield and Birdsall 1998), and we did not perform extensive pharmacological studies to identify the subtype of muscarinic receptor involved in the inhibition of the EPSC. We found that pirenzepine, which acts predominantly on M1 and M4 receptors, mimicked much of the effects of atropine. These results are in agreement with those of Pennartz and Lopes da Silva (1994). Others have suggested that muscarinic receptor-mediated inhibition in the nAcb and dorsal striatum were mediated by M1 (Hsu et al. 1995; Sugita et al. 1991) or M1-M4 (Hernandez-Echeagaray et al. 1998) receptors. A subset of M1, M3, and M4 muscarinic receptors are found on axon terminals forming asymmetrical synapses (Hersch and Levey 1995; Hersch et al. 1994) and provide an anatomical basis for the presynaptic modulation of glutamatergic neurotransmission by ACh. In contrast, the M2 receptor appears to be located on axon terminals making symmetrical synapses, suggesting that they do not participate in the modulation of excitatory input. M1 and M4 receptors mRNA are found in cortical and hippocampal pyramidal neurons as well as in the amygdala and thalamus (Buckley et al. 1988; Wei et al. 1994), and these structures could be the source of presynaptic muscarinic receptors located on glutamatergic terminals in the nAcb.

**Nicotinic potentiation of EPSCs**

ACh and CCh not only act on muscarinic receptors but also activate nicotinic receptors. Under the present experimental conditions, nicotinic receptor-mediated excitation became apparent only when appropriate muscarinic receptor antagonists were added to the superfusing medium, suggesting that nicotinic receptor-mediated excitation was masked by a predominant muscarinic inhibition. Furthermore, the application of DMPP mimicked the enhancing effects produced by general cholinergic agonists in the presence of atropine or pirenzepine, and this effect was blocked by mecamylamine, a specific nicotinic receptor antagonist, showing that the potentiation of the EPSC was mediated by the activation of nicotinic receptors. To our knowledge, this is the first demonstration that glutamatergic neurotransmission is modulated by nicotinic presynaptic receptors in the nAcb.

The presence of presynaptic nicotinic cholinergic receptors has been documented in both the nAcb and dorsal striatum (see Lendvai and Vizi 1999; MacDermott et al. 1999). Our findings are in agreement with several recent studies showing that the activation of presynaptic nicotinic cholinergic receptors facilitates glutamatergic neurotransmission in different regions of the CNS (e.g., Aramakis and Metherate 1998; Girod et al. 2000; Gray et al. 1996; Guo et al. 1998; McGehee et al. 1995). These studies suggested that facilitation of glutamatergic neurotransmission was mediated by nicotinic receptors containing the α subunit. Our results suggest that another type of nicotinic receptor is involved in the nucleus accumbens because receptors containing the α subunit are insensitive to mecamylamine (e.g., MacDermott et al. 1999). Our results are supported by recent findings showing that nicotine increases glutamate release in the nAcb via a mecamylamine-sensitive nicotinic receptor (Reid et al. 2000).

Several studies have demonstrated that local nicotinic receptor activation increased dopamine release in the nAcb (Fu et al. 2000; Hildebrand and Svensson 2000; Nisell et al. 1994a,b) raising the possibility that some of the effects we observed were indirectly mediated through the dopaminergic system. This appears unlikely because nicotinic-evoked dopamine release in the nAcb has been found to be insensitive to mecamylamine but is sensitive to α subunit antagonists (Fu et al. 2000), suggesting that a different type of nicotinic receptors control glutamate and dopamine release in the nAcb.

We have found that nicotinic agonist enhanced both AMPA/KA and NMDA receptor-mediated EPSCs but that the effect was statistically larger on NMDA than on AMPA/KA-mediated response. This is in partial agreement with Aramakis and Metherate (1998), who found that in rat auditory cortex during postnatal development nicotine selectively enhanced NMDA receptor-mediated EPSP while producing no change in AMPA/KA receptor-mediated EPSP. The authors concluded that nicotinic receptors were located on glutamatergic terminals at synapses containing only NMDA receptors, whereas the present results suggest that nicotinic receptors are located on
territory effects of ACh increased during the postnatal period possibly contributes to the maturation and refinement of the glutamatergic innervation of the nAcb. In addition, glutamatergic innervation of the nAcb is topographically organized and ACh could participate in the refinement of this organization by turning on and off specific inputs in the nAcb.

Disruption of some of the glutamatergic inputs to the nAcb during early postnatal development (P7) has been found to produce enduring behavioral changes (Al Amin et al. 2001; Flores et al. 1996a,b; Lipska et al. 1993; Sams-Dodd et al. 1997; Weinberger and Lipska 1995; Wood et al. 1997; see also Lipska et al. 1998) as well as changes in dopaminergic receptors (Baca et al. 1998; Flores et al. 1996a,b) and dopamine release (Lillrank et al. 1999) in the nAcb. Typically, these changes are expressed only after puberty, and, interestingly, the same lesions at P14 or in adult animals produced no comparable changes (Wood et al. 1997), suggesting that there is a critical period during which developmental plasticity can be expressed in the nAcb.

We are grateful to Dr. Arlette Kolta for commenting on an earlier version of the manuscript.

This work was supported by funds from the Canadian Institutes for Health Research (Grant MT-14820) and the National Science and Engineering Research Council of Canada (Grant OGP 184095). R. A. Warren was supported by a fellowship from Fonds de la Recherche en Santé du Québec.

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